The Relationship Between Intracellular Phosphate, Protonmotive Force, and the Energy Spilling Rate of *Streptococcus bovis* JB1

D.R. Bond and J.B. Russell

Introduction

The growth efficiency (yield) of bacteria is generally much lower than the amount that would be predicted from growth and maintenance, and previous work indicated that the ruminal bacterium, *Streptococcus bovis*, was able to spill large amounts of energy via a futile cycle of protons through the cell membrane. The energy spilling of ruminal *S. bovis* was greatest when amino nitrogen was not available, but the regulation was not understood. Because mixed ruminal bacteria behave in a similar fashion, we decided to study the energy spilling of *S. bovis* in greater detail.

Materials and Methods

S. bovis JB1 was grown anaerobially on glucose in batch and continuous cultures in a medium that had ammonia or ammonia plus Trypticase as a nitrogen source. Cell suspensions were centrifuged through silicone oil, treated with perchloric acid, and assayed spectrophotometrically for fructose-1,6-diphosphate (FDP). Intracellular phosphate was assayed via an assay employing ammonium heptamolybdate, malachite green, and Sterox color reagent. The pH gradient across the cell membrane (ΔpH) and the electrical potential ($\Delta \Psi$) were determined by methods employing silicon oil centrifugation, the distribution of 3H-tetraphenylphosphonium bromide (3H-TPP^+) and ^{14}C-benzoate across the cell membrane, and the Nernst equation ($-2.3\ RT/\mathcal{F} \times \log$ [concentration in]/[concentration out]). ATP was assayed with a luminometer using luciferin-luciferase. Fermentation acids were analyzed by high-pressure liquid chromatography. Glucose was determined via hexokinase and glucose-6-phosphate dehydrogenase. Protein was determined by the Lowry method.

Results

When the rate of glucose addition to non-growing *S. bovis* cell suspensions was increased, the fermentation was homolactic, fructose-1,6-diphosphate (FDP) increased, intracellular phosphate (P_i) declined and the energy spilling rate increased. ATP and ADP were not significantly affected by glucose consumption rate, but the decrease in P_i was sufficient to cause an increase in the free energy of ATP hydrolysis ($\Delta G'p$). The increase in $\Delta G'p$ was correlated with an increase in protonmotive force (Δp). *S. bovis* continuous cultures (dilution rate of 0.65 h^{-1}) that were provided with ammonia as the sole nitrogen source also had high rates of lactate production and energy spilling. When Trypticase was added as a source of amino acids, lactate production decreased, a greater fraction of the glucose was converted to acetate, formate and ethanol, and the energy spilling rate decreased. Trypticase also caused a decrease in FDP, an increase in P_i and a decrease in Δp. The change in Δp could be explained by P_i-dependent changes in the free energy of ATP hydrolysis ($\Delta G'p$). When P_i declined, $\Delta G'p$ and Δp increased. The ratio of $\Delta G'p$ to Δp (mV/mV) was always greater (> 4) at low rates of energy spilling, but declined when the energy spilling rate increased. Based on these results, it appears that Δp and the energy spilling rate are responsive to fluctuations in the intracellular phosphate concentration.

Discussion

Previous work indicated that amino acid limitation (due to growth on ammonia nitrogen) increased the energy spilling rate of *S. bovis* energy-excess batch cultures, and the present experiments indicated that amino nitrogen was also able to regulate the energy spilling rate of energy-limited continuous cultures. Other workers reported that bacteria growing in rich media had lower Δp values than bacteria growing in minimal media, but a relationship between Δp and energetic efficiency was not considered. When *S. bovis* continuous cultures were supplemented with a source of amino acids (Trypticase), Δp and energy spilling both declined.

The energy spilling rates of growing and non-growing *S. bovis* cells could be correlated with a decline in FDP and increase in intracellular P_i. When P_i increased, both the $\Delta G'$ of ATP hydrolysis and the Δp declined. Creation of the Δp is driven by the $\Delta G'$ of ATP hydrolysis, and some researchers have assumed that Δp is in equilibrium with $\Delta G'p$. However, the cell

membrane is not a perfect insulator. If proton flux into the cell is rapid (e.g. high rates of energy spilling), Dp should be less than the amount predicted by DG'p. When *S. bovis* was spilling energy at a slow rate, the ratio of DG'p to Dp was greater than 4, but this ratio declined to 3.3 when the energy spilling rate was high. Other workers have noted a similar variation. The DG'p to Dp of *Lactococcus lactis* ranged from 3 to 4.3, and the DG'p to Dp of *Lactococcus cremoris* ranged from 4.5 to 2.

Previous work indicated that the energy spilling reaction of *S. bovis* required a decrease in membrane resistance and an increase in proton conductance. Because the non-growth energy dissipation rate was as high as 70 mmol ATP/g protein/h and the H+/ATP stoichiometry of the F_1F_0 ATPase can be high as 4, the proton permeability of *S. bovis* could be as high as 280 mmol H^+/g protein/h. The passive proton permeability of *L. lactis* is approximately 1.6 µS/cm^2 (approximately 1.5 mmol H^+/g protein/h at a Dp of -120 mV). Mammalian mitochondria have ion channels that can increase non-growth energy dissipation, but flux through these channels decreases Dp. *S. bovis* cells had higher (not lower) Dp when rates of energy spilling were high. The bacterial protein, colicin E1, is a Dp-dependent (voltage-gated) ion channel, with a threshold of approximately 80 mV. Further work is needed to see if *S. bovis* uses a similar mechanism to regulate membrane resistance and energy spilling rate.

Conclusions

The energy spilling rate of *S. bovis* is regulated by an elegant cascade of metabolic changes that ultimately change the resistance of the membrane to protons. This regulation can be triggered by a deficiency of amino nitrogen.